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HEADSPACE GAS CHROMATOGRAPHY OF STIMULANTS IN URINE BY IN-COLUMN TRIFLUOROACETYL DERIVATIZATION METHOD

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SUMMARY

The analysis of stimulants in urine using a headspace gas chromatography system equipped with an in-column sample trifluoroacetylation unit was investigated. A 5-ml aliquot of urine containing stimulants was pipetted into a 20-ml autosampler vial together with 3.5 g of potassium carbonate. The vial was sealed and heated for 20 min at 80°C, then 0.8 ml of the headspace gas and N-methylbis(trifluoroacetamide) gas were injected simultaneously into the gas chromatograph equipped with a flame ionization detector and a fused-silica capillary column (DB-1, 30 m × 0.32 mm I.D., film thickness 0.25 μm), with a gas-tight syringe. Calibration graphs prepared by the absolute calibration curve method showed good linearity over the concentration range of 0.04 to 50 μg/ml for methamphetamine hydrochloride and amphetamine sulphate. The detection limits were 0.03 μg/ml for both of these compounds.

INTRODUCTION

In recent years, for clarifying methamphetamine use, the number of urine samples obtained from methamphetamine abusers has increased strikingly. For this reason, an automated, rapid and accurate method was required.

We have previously reported an automated analysis method for stimulants in urine, using the technique of headspace gas chromatography (HSGC)[1]. This technique requires no sample pretreatment such as liquid–liquid extraction of the target compounds. An urine sample is placed in a vial, made alkaline and subjected to HSGC. Compared with the conventional GC method which utilizes solvent extraction, this technique provides a more rapid analysis and higher sensitivity. The principal advantage was that the column was not contaminated at all by the urine components.

It was thought that the on-line connection of a mass spectrometer in HSGC would provide more reliable qualitative information, but the stimulants detected as free bases did not give any molecular ions (M^+) in electron-impact (EI) ionization mass spectrometry, only low-molecular-weight fragment ions produced by fragmen-

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tation at the β-position[2]. Our presumption was that derivatization with trifluoroacetyl (TFA) would provide higher detection sensitivity because of the higher vapour pressures of the derivatives[3–5].

In this report, a newly developed system which ensures higher sensitivity and selectivity is presented. An inlet device to introduce the TFA derivatizing reagent was attached to the injection port of the gas chromatograph. N-Methylbis(trifluoroacetamide) (MBTFA)[6], which is a moderate TFA derivatizing reagent for amines and phenols, was used for derivatization. The stimulants in the form of free bases were introduced by the headspace method, derivatized in the capillary column, chromatographed and detected as TFA derivatives.

One noteworthy advantage of this method is that the operator is not required to handle organic solvents or urine samples for pretreatment.

EXPERIMENTAL

Materials

Methamphetamine hydrochloride and amphetamine sulphate were obtained from Dainippon (Osaka, Japan) and Takeda (Osaka, Japan), respectively. MBTFA was obtained from Wako (Osaka, Japan). Other reagents were of analytical grade.

A glass vial (20 ml), poly(tetrafluoroethylene) (PTFE) septum and aluminium vial cap were obtained from Maruemu (Osaka, Japan).

The standard sample solutions, 0.01–200 μg/ml, were prepared by adding the salts of methamphetamine hydrochloride and amphetamine sulphate to urine samples obtained from healthy volunteers who had not taken any drugs. The MBTFA reagent was used without dilution.

Instrumentation

GC was carried out on a Shimadzu GC-9A gas chromatograph equipped with a CLH-702 split type sample injector, a flame ionization detector, a TFA derivatizing device, an HSS-2A headspace sampler and a C-R3A Chromatopac data processor. The column used was a DB-1 (J&W Scientific) fused-silica capillary column, 30 m × 0.32 mm I.D., film thickness 0.25 μm. The injection and detector temperatures were both 260°C, and the column temperature was 130°C. The linear velocity of the carrier gas (nitrogen) was 50 cm/s.The sample heating temperature was 80°C, the sample heating time was 20 min and the gas-tight syringe temperature was 130°C.

Adaptation of headspace sampler

The HSS-2A permitted automatic sampling and headspace gas injection of up to 40 samples placed on the carousel.

The gas-tight syringe was rinsed with purge gas (nitrogen) at temperatures of 40–150°C. This method of syringe washing was not satisfactory for the analysis of stimulant amines having high boiling points, and therefore the syringe was washed as follows: a vial containing water was placed between sample vials and heated to 80°C by the vial heating block; the syringe was washed five times with hot water and completely dried by pumping 15 times with purge gas. The syringe temperature was set at 130°C.

Device for in-column TFA derivatization

Fig. 1. shows the construction of the device for TFA derivatization. A 1-ml volume of MBTFA solution was placed in a 10-ml consolidated glass vial and nitrogen introduced into the solution at a rate of 20 ml/min. The MBTFA gas evaporated was introduced into the 1-ml sample loop connected to the six-way valve set at the position shown by the dotted line. Just after the headspace gas was introduced, the six-way valve was switched to the position indicated by the solid line. Thus, the stimulants were derivatized on the column.

Method of analysis

An urine sample (5 ml) was placed in a vial and 3.5 g of potassium carbonate were added. The vial was sealed with a septum (PTFE) and an aluminium cap, and placed on the carousel. (All the above operations were carried out manually.) Then the operational sequence of the HSS-2A was started. The carousel rotated and the sample vial was placed into the vial heating block. The vial was kept at 80°C for 20 min, until the vapour pressure in it had equilibrated. A 0.8-ml volume of the headspace gas was taken by the gas-tight syringe and injected for GC. The six-way valve of the TFA derivatizing device was switched to inject the MBTFA reagent.

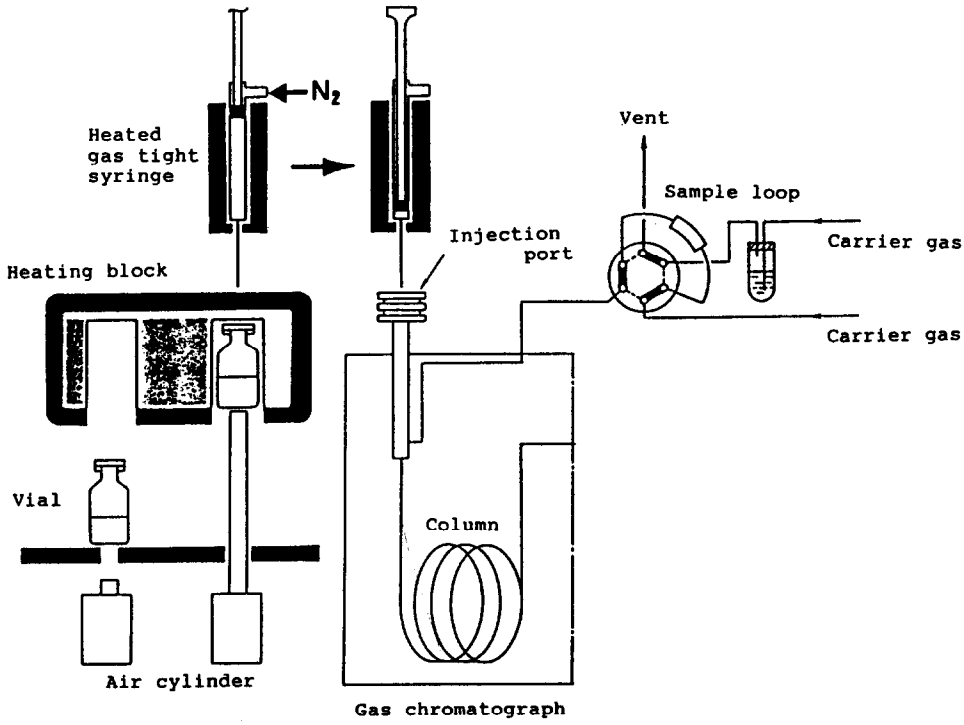

Fig. 1. Flow diagram of the in-column TFA derivatization HSGC system.

RESULTS AND DISCUSSION

Sample heating temperature and time

A 5-ml aliquot of each standard 10 μg/ml aqueous solution containing methamphetamine hydrochloride, amphetamine sulphate and 3.5 g of potassium carbonate was placed in a sample vial. The sample vials were heated for 20 min at 40, 50, 60, 80 or 90°C in the vial heating block, and 0.8 ml of the headspace gas were taken and injected for GC together with the TFA derivatizing reagent. The amounts of methamphetamine and amphetamine were related to the peak areas by means of the C-R3A data processor.

The results showed that a higher sample heating temperature gave a larger peak area. The peak area became constant at temperatures in the rang 80–90°C. In subsequent experiments, therefore, the sample heating temperature (vial heating block temperature) was set at 80°C.

The influence of the sample heating time was similarly examined and a constant value of 20 min at 80°C was found to be optimum.

Injection volume of MBTFA reagent

The volume of the MBTFA solution for effective derivatization was investigated using sample loops of different sizes. A 5-ml volume of the standard solution, containing 20 μg/ml of methamphetamine hydrochloride and amphetamine sulphate and 3.5 g of potassium carbonate was placed in a vial, and the vial was sealed and kept at 80°C for 20 min. Derivatization HSGC was carried out using sample loops of 1, 2 and 5 ml in volume. Use of the 1-ml sample loop for methamphetamine hydrochloride and amphetamine sulphate gave only the peak of the derivative and no peaks due to stimulants in their free basic states.

Addition of sodium hydroxide

In this method, an urine sample was made alkaline in the vial and the released gases of the stimulants were chromatographed after TFA derivatization. In our previous study[1], sodium hydroxide was used for alkalization and potassium carbonate for salting out. Since a certain amount of potassium carbonate can alkalize samples and, at the same time, serve as a salting-out agent, the addition of sodium hydroxide was considered unnecessary.

The effect of the addition of sodium hydroxide was investigated using standard solutions of 1, 5, 10 and 50 μg/ml, to 5 ml of which (1) 3.5 g of only potassium carbonate were added, and (2) 3.5 g of potassium carbonate and 0.5 ml of a 10% solution of sodium hydroxide were added. These samples were analyzed by HSGC after TFA derivatization. The addition of sodium hydroxide had no significant effect on the peak areas. In subsequent experiments, only potassium carbonate was added to urine samples.

Addition of potassium carbonate

The amount of potassium carbonate added was investigated. The technique of HSGC was generally applied to analyses of volatile components because heating in sample vials might not satisfactorily evaporate stimulant components which have high boiling points. It was investigated whether the evaporation efficiency can be enhanced by the salting-out effect employing potassium carbonate.

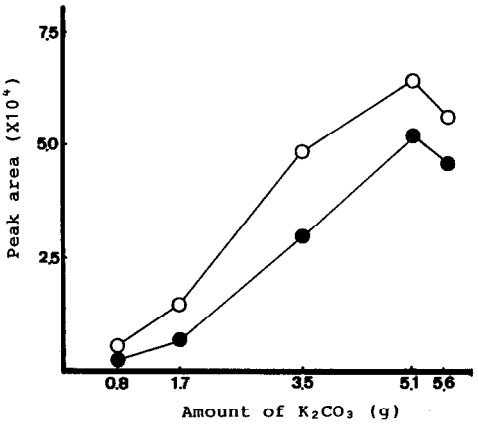

Fig. 2. Effect of potassium carbonate addition to the sample solution: ● = amphetamine; ○ = methamphetamine. Each point respresents the mean of three experimental values.

A 5-ml aliquot of each of standard 10 μg/ml aqueous solution of methamphetamine hydrochloride and amphetamine sulphate was placed into a sample vial: to each of them was added, respectively, 0.8, 1.7, 3.5, 5.1 and 5.6 g of potassium carbonate. The vials were allowed to stand at 80°C for 20 min, and the headspace gases were chromatographed after TFA derivatization. The results shown in Fig. 2 indicate significant increases in response or peak area over the range of salt added. A saturation point was reached when 5.6 g had been added, after which the sensitivity for both methamphetamine and amphetamine decreased. The appropriate amount of potassium carbonate was concluded to be *ca.* 3.5 g, the dissolution time and other factors remaining constant.

Sample volume

In HSGC the concentrations of the components in the gas phase are determined by their concentrations in the liquid phase, and do not depend on the volume of the solution[7]. In our method, however, since the salting out effect of potassium carbonate is utilized, the influence of the sample volume was investigated. Into 20-ml sample vials, 2.5, 5.0, 7.5 and 10 ml of standard 10 μg/ml aqueous solutions of methamphetamine hydrochloride and amphetamine sulphate were placed. To each of these, 0.7 g potassium carbonate were added per ml (which corresponds to 3.5 g per 5 ml of sample solution, when employing the standard volume in our method); the vials were sealed and allowed to stand at 80°C for 20 min, and the contents derivatized. The results are shown in Fig. 3. The peak areas for methamphetamine and amphetamine increase in proportion to the sample volume. A suitable sample volume was 5 ml, by consideration of the sample volumes required for other methods of analysis. The same investigation was repeated with no potassium carbonate added, and the solution made alkaline with 0.5 ml of 10% sodium hydroxide. The vials were then sealed and allowed to stand at 80°C for 20 min. The headspace gases were chromatographed after derivatization. There was no change in peak areas attributable to the change in sample volume. This shows that the addition of potassium carbonate results in vaporization of most stimulants in the solution and does not give gas–liquid equilibrium as is always the case in headspace analysis.

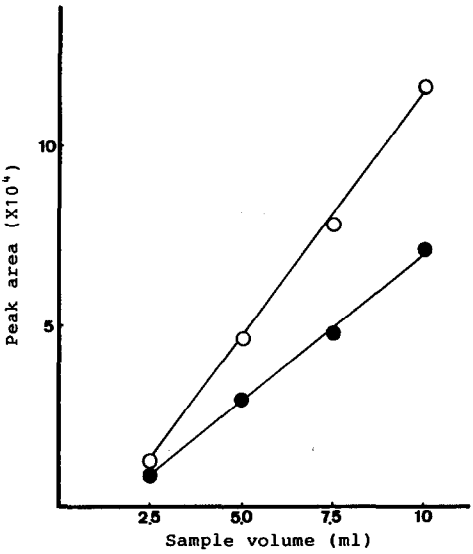

Fig. 3. Effect of sample volume: ● = amphetamine; ○ = methamphetamine. Each point represents the mean of three experimental values.

Calibration graph

Aliquots of 5 ml of standard solutions of methamphetamine hydrochloride and amphetamine sulphate were added to 5 ml of urine to give drug solutions in the concentration range of 0.01–100 μg/ml. To each of these, 3.5 g of potassium carbonate were added. Each solution was heated at 80°C for 20 min. The headspace gases were analyzed by HSGC after TFA derivatization. The calibration graphs were constructed using the absolute calibration curve method. They were linear within the concentration range from 0.04 to 50 μg/ml. Fig. 4 shows calibration graphs for methamphetamine hydrochloride and amphetamine sulphate. The detection limit is 0.03 μg/ml for both of these compounds. The detection limit in HSGC of non-derivatized stimulants is 0.07 μg/ml for both methamphetamine and amphetamine[1]. The coefficient of variation obtained for eleven replicate analyses of the standard 10 μg/ml solution was 2.36% for methamphetamine and 2.38% for amphetamine.

Deterioration of urine sample

The deterioration of the urine sample was investigated before the addition of potassium carbonate.

Standard 20 μg/ml aqueous solutions of methamphetamine hydrochloride and amphetamine sulphate were allowed to stand at room temperature for 1, 5, 10 and 20 days. Then, to 5 ml of each of these solutions were added 3.5 g of potassium carbonate and the headspace gas was chromatographed after being TFA derivatized. The results are shown in Fig. 5. No change in concentration of methamphetamine or amphetamine was observed in the samples that were allowed to stand for up to 5 days. In the case of samples allowed to stand for 10 days, the concentrations of both methamphetamine and amphetamine decreased to 96% of the initial concentrations, and in the case of 15-days-old samples the methamphetamine decreased to 86% and the

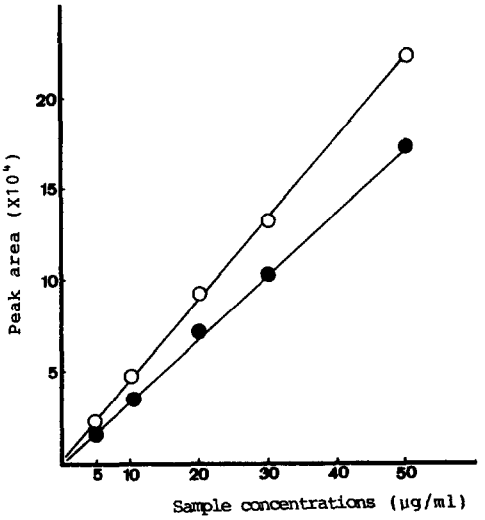

Fig. 4. Calibration graphs for amphetamine (●) and methamphetamine(○). The coefficient of variation for the 10 μg/ml sample was 2.36% for methamphetamine and 2.38% for amphetamine ($n=11$).

amphetamine to 94%. In the case of 20-days-old samples, the methamphetamine decreased to 70% and the amphetamine to 86%. It is recommended, therefore, to analyze urine samples before they deteriorate or to keep them in a refrigerator.

The analysis of deteriorated blank urine samples gave no interference peaks having retention times close to those of stimulants or similar compounds.

Comparison with solvent extraction GC method

The data obtained by the present method were compared with those obtained by the GC method in which the target compounds were extracted with organic solvents. The urine samples were taken from five suspects. In the present method, 3.5 g of

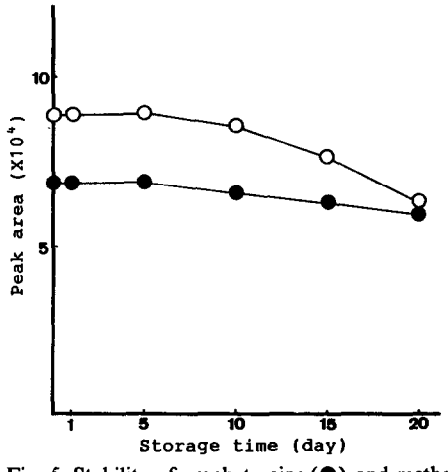

Fig. 5. Stability of amphetamine (●) and methamphetamine (○) in solution. Each point represents the mean of three experimental values.

TABLE I

COMPARISON OF GC AND HSGC FOR AMPHETAMINE AND METHAMPHETAMINE DETERMINATION IN URINE

GC method: 3 ml of urine were alkalized with ammonia and extracted with chloroform. HSGC method: 5 ml of urine and 3.5 g of K_2CO_3 were placed in a 20-ml vial. The solution was allowed to stand at 80°C for 20 min. TFA derivatized and analyzed by HSGC. Values given in μg/ml.

No.	*Amphetamine*		*Methamphetamine*	
	GC	*HSGC*	*GC*	*HSGC*
1	0.5	0.46	2.77	2.49
2	2.16	2.27	6.74	6.38
3	1.36	1.39	14.09	14.65
4	1.97	2.05	14.57	15.01
5	4.97	5.21	39.51	42.73

$r = 0.999$ ($p < 0.01$)

potassium carbonate were added to 5 ml of urine sample and the headspace gas was chromatographed after TFA derivatization. In the GC method, 3 ml of urine sample were alkalized with ammonia, and the target compounds were extracted into 1 ml of chloroform. The quantitation was carried out using the predetermined calibration graphs. Table I shows the corresponding data.

The correlation coefficient between the quantitative data of methamphetamine and amphetamine obtained by the two methods was 0.999 ($p < 0.01$). The values given by the two methods are in good agreement. Fig. 6 shows a typical gas chromatogram of a suspect urine sample.

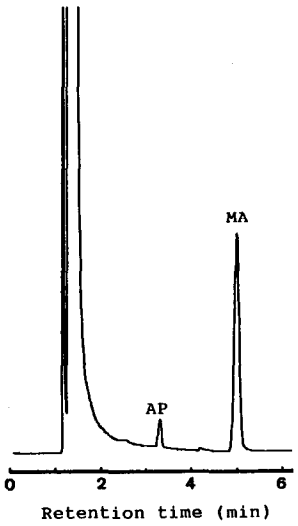

Fig. 6. Headspace gas chromatogram of trifluoroacetylated amphetamine (AP) and methamphetamine (MA) in urine. GC conditions: DB-1 fused-silica capillary column, 30 m × 0.32 mm I.D., film thickness 0.25 μm; injection temperature 260°C, column temperature 130°C. Sample: 5 ml of urine + 3.5 g of K_2CO_3 at 80°C for 20 min.

TABLE II

COMPARISON OF VARIOUS SCREENING METHODS FOR METHAMPHETAMINE IN URINE

− = Negative; + = positive. TBPE method: TBPE was added to 5 ml of urine. Simon's method: 5 ml of urine were alkalized with ammonia, and extracted with chloroform–isopropanol mixture. GC–MS method: 5 ml of urine were alkalized with ammonia, extracted with chloroform–isopropanol mixture and PFP derivatized. Present method: 5 ml of urine and 3.5 g of K_2CO_3 were placed in a 20-ml vial; the solution was allowed to stand at 80°C for 20 min, TFA derivatized and analyzed by HSGC.

TBPE method	*Simon's reagent*	*GC–MS*	*Present method*	*Number of specimens*
+	+	+	+	28
+	−	−	−	8
−	−	+	+	6
−	−	−	−	8

Comparison with conventional method of analysis

In Japan, urinary stimulants are determined in the following manner: screening test by the tetrabromophenolphthalein ethyl ester (TBPE) method, extraction of stimulants into solvent, Simon's reaction[8] and then determination by infrared spectrophotometry or gas chromatography–mass spectrometry (GC–MS). The reliability of the present method was investigated in the following way: Fifty suspect urine samples were (1) screened by the TBPE method, (2) alkalized with ammonia, subjected to extraction with chloroform–isopropanol (3:1, v/v), the solvent removed and the residue subjected to Simon's reaction; (3) some part of the sample was treated as described in (2) and derivatized with pentafluoropropionyl (PFP), and then the derivative was analyzed by GC–MS. The results were compared with those given by the present method (Table II). The sample size was 5 ml for both methods.

The present method and the GC–MS method gave the same results for 50 samples out of 50 (100% agreement), the screening method (TBPE method) and the GC–MS method for 36 samples (70% agreement) and Simon's method and the GC–MS method for 44 samples (88% agreement). These data show the high reliability of the present method.

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